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ORIGINAL PAPER

Effect of electrostatic field on seed germination and seedling growth of *Sorbus pohuashanesis*

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Abstract: A study was conducted to determine the effects of electrostatic field (ESF) treatment on seed germination and seedling growth of *Sorbus pohuashanesis*. The experiments were arranged by uniform design computed by the Data Processing System (DPS), including three levels of seeds soaking time, four levels of ESF intensity and four levels of ESF treatment time, with 12 treatments. Ten seeds were used in each treatment with three replicates. Seed vigor, seed germinating ability, emergence rate of seedling, survival rate of seedling, and seedling height and diameter, as well as the change in activities of superoxide dismutase (SOD), soluble protein contents, total chlorophyll contents, soluble total sugar contents in leaves of *S. pohuashanensis* seedlings were measured after ESF treatments. The experiment results show that ESF treatment could improve the water absorption ability of dry seeds of *S. pohuashanensis,* resulting in fast germination at room temperature under light conditions. Combined treatment of ESF with cold stratification could increase seed germination percentage significantly (to 42.20%), promote seedling height growth, affect leaf SOD activity, and could raise contents of total chlorophyll, soluble protein, and total soluble sugar in leaves. Seed soaking time had a significant effect on seed relative electroconductivity, seed germination under light, SOD activity, soluble protein content and total soluble sugar content of seedling leaves. ESF intensity exerted a moderate effect on these indexes. ESF treatment time only had significant effect on total chlorophyll contents, no evident effect on other indexes.

Keywords: *Sorbus pohuashanesis*; seed germination; seedling growth; electrostatic treatment

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Introduction

Downy Chinese mountain ash (*Sorbus pohuashanensis* (Hance) Hedl) is a deciduous small tree species in genus *Sorbus* of Rosaceae distributed in North China, Northeast China, Northwest China, North Korea and the Far East Area of Russia. With graceful foliage, showy flowers and bright-red colored fruits, *S. pohuashanensis* is a popular candidate species for ornamental purpose (Mu 2001). This species was widely planted in the northern area with cold and rigid environment conditions due to its strong tolerance to cold environment (Ren 1985), poor soil condition, diseases, and pollution. However, except for the scattered growing in some parks and courtyards, few mountain ash trees were planted in China. The most important reason is the difficulty in the propagation of this species. The limited propagation experience indicates that the seed rate of *S. pohuashanensis* fruits is only 1%（w/w） and the seed germination percentage is only about 10%. Like other mountain ash species described by Taylor and Gerrie (1987), Oster et al. (1987), Yagihashi et al. (1998) and Paulsen et al. (2002), *S. pohuashanensis* seeds could only germinated when the fruits were predated by birds or other animals under natural conditions or cold stratification for 2–4 months or more in nursery production (Zhou 1986). Even if the seeds are treated, the germination percentage is far away from desired. Operationally, over sowing was a commonly used way to obtain more seedlings, which resulted in an unwilling waste of manpower, materials and economic cost.

Traditionally, seeds are treated with chemicals to promote germination; however, chemical treatment is quite expensive and potentially harmful to soil. Researches demonstrated that the exposure to a high-intensity electrostatic field (ESF) could be an effective substitute for the chemical treatments. Seeds of many crop and tree species, when properly treated with high-intensity ESF, showed an increase in seed viability, germination percentage and subsequent growth (Hou et al. 1995; Bi et al. 1995; Li et al. 1996; Cai et al. 2003; Morar et al. 1999; Sidaway 1969 and 1970). In the present study, mature seeds of *S. pohuashanensis* were treated by high-intensity ESF, and then the effects of

high-intensity ESF on seed germination and seedling growth as well as the relative importance of each factor (soaking duration, ESF intensity and ESF treatment duration) were investigated.

Materials and methods

Plant materials

S. pohuashanensis fruits were collected in late September at the Wuying National Forest Park in Yichun City, Heilongjiang Province, China. After extraction from fruits, seeds were air dried and cleaned, and then the seeds in a sealing bag were stored under 0–5°C in refrigerator. The mean moisture content of the seeds was 5.71%. The 1000-seed weight was (2.39±0.01) g. Seed viability by tetrazolium staining test (ISTA 1996) was 88.89%.

Electrostatic field treatments

The ESF used was parallel plank with a lowest intensity of 12 kV, and the distance between the planks could be adjusted to 5 cm, 10 cm, 15 cm and 20 cm.

The pretreatment of seeds involved no soaking, soaking for 1 day or soaking for two days. Then, the seeds were put between each flat panel in the ESF dispersively. Plank distance of 10 cm was chosen, and the treatment of different ESF strength and different duration were conducted. The ESF strength ranges from 100 to 250 $kV\cdot m^{-1}$, and the duration ranges from 5 min to 40 min. The experiments were arranged according to the experimental scheme given by uniform design computed by the Data Processing System (DPS). DPS was developed by Tang and Feng (2002). As shown in Table 1, the scheme contained three levels of seeds soaking time, four levels of ESF intensity and four levels of ESF treatment time, resulting in 12 treatments (the scheme was replicated five times by the DPS，and the smallest even deviation was elected, that was 0.155). Ten seeds were used in each treatment, and each experiment was replicated three times.

Table 1. Experimental scheme of electrostatic field treatments for seeds of *S. pohuashanensis*

Treatment No.	Water soaking time (days)	ESF intensity (kV·m-1)	ESF treatment time (min)
T1	0	100	28
T2	0	150	17
T3	0	200	40
T4	0	250	5
T5	1	100	5
T6	1	150	28
T7	1	200	17
T8	1	250	40
T9	2	100	40
T10	2	150	5
T11	2	200	28
T12	2	250	17

The water absorbing ability of seed

ESF treated seeds (2 g) and controlled dry seeds were respectively soaked in distilled water for 48 h, absorbed water on surface by filter paper, weighed and calculated the water absorbing quantity of seeds. The experiment was replicated three times.

The measurement of relative electroconductivity

Fifty controlled seeds (3 patterns as without soaking, soaking for 1 day and soaking for 2 days) and 50 ESF treated seeds (three patterns same as controlled seeds) were first rinsed with double distraction water, absorbed water on surface by filter paper, and soaked in flasks with 20 mL double distraction water for 48 h at 25°C. The electroconductivity of the leachate solution was tested and recorded as S_1. After S_1 tested, the leachate solution together with the seeds was put into boiled water for 20 min, cooled down to 25°C, tested the electroconductivity and recorded as S_2. The electroconductivity of double distraction water was defined as the control and recorded as S_0. Each test was replicated three times. The relative electroconductivity was computed as:

$$S = \frac{S_1 - S_0}{S_2 - S_0} \times 100\% \qquad (1)$$

Germination tests and pregermination treatments

Germination test under light

Both controlled seeds and ESF treated seeds were first soaked in water for two days, and disinfected for 2 h by 5 $g\cdot L^{-1}$ $KMnO_4$, followed by rinse in autoclaved distill water, then germinated under light at 25°C. One hundred seeds were used in each Petri dish and three Petri dishes were used for each treatment.

Cold stratification

Disinfected seeds were mixed with wet sand (the moisture content was about 80%) in a ratio of 1:3 (v/v). Then the mixture was put in 0–5°C for pregermination. Three replicates were used in each treatment.

The tests lasted for 200 days from May 14 to November 30.

Sowing tests of the germinated seeds

Germinated seeds of *S. pohuashanensis* were sowed into disinfected growth medium **(**peat/vermiculite=2/1, v/v**),** covered with 1 cm disinfected fine soil, watered, and cultured in green house under 17–22°C. After three to four days of seedlings emerged, the cover was removed and air was allowed to convect. Seedling emerging state in 10 days of culture was recorded. The growth medium was watered regularly and kept wet. In 55 days of culture when seedlings emerged as four leaves with a curly leaf, the survival rate, shoot height, and root-collar diameter were recorded.

Physiological indexes tests of seedlings

Superoxide dismutase (SOD) activity was tested by nitroblue tetrazolium (NBT) photoreaction method, expressed as $U\cdot mg^{-1}$ protein. Soluble protein content was measured according to the

method described by Liu (1994), protein content in 1 g fresh leaves expressed as mg·g^{-1} FW. Chlorophyll content and total soluble sugar content were tested according to the methods described by Wang et al. (2003). The contents of soluble protein, chlorophyll and total soluble sugar in leaves were expressed as mg·g^{-1} FW.

Statistical analysis

Germination was calculated as a percentage of all seeds (viable and dead) in the test. To improve normality and homogeneity of variances, data concerning percentage were arcsine transformed before analysis. All statistical analyses were performed with DPS (Data Processing System 7.05; Tang and Feng. 2002).

Results

The experiment results by uniform design statistically analyzed by DPS are shown in Table 2.

Table 2 Effects of electrostatic field treatments on seed germination and seedling growth of *S. pohuashanensis*

No.	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
T1	0	100	28	58.51 ± 5.54^{ABC}	49.7 ± 7.02^{AB}	1.33 ± 1.53^{a}	53.0 ± 5.66^{a}	29.02 ± 1.65^{EFG}	24.53	89.23
T2	0	150	17	62.32 ± 2.75^{A}	44.0 ± 3.61^{ABC}	2.00 ± 1.00^{a}	51.0 ± 8.49^{a}	38.03 ± 2.18^{AB}	11.54	90.48
T3	0	200	40	58.47 ± 6.52^{ABC}	47.0 ± 1.14^{AB}	4.33 ± 5.77^{a}	53.0 ± 5.66^{a}	42.20 ± 2.19^{A}	20.41	86.08
T4	0	250	5	59.90 ± 3.64^{AB}	44.0 ± 5.57^{ABC}	0.67 ± 1.00^{a}	47.0 ± 2.83^{ab}	34.57 ± 3.38^{BCD}	25.49	96.97
T5	1	100	5	38.57 ± 11.54^{BCD}	47.5 ± 6.36^{AB}	0.67 ± 5.77^{a}	48.0 ± 5.66^{a}	32.16 ± 3.20^{CDE}	15.63	73.33
T6	1	150	28	34.93 ± 8.96^{D}	46.7 ± 1.69^{AB}	1.33 ± 0.58^{a}	52.5 ± 4.95^{a}	25.32 ± 1.16^{FGH}	16.00	75.00
T7	1	200	17	42.30 ± 5.11^{ABCD}	38.7 ± 3.06^{ABCD}	3.00 ± 2.65^{a}	48.0 ± 3.21^{a}	23.10 ± 0.99^{H}	13.5	100.00
T8	1	250	40	36.34 ± 12.48^{CD}	51.7 ± 0.58^{A}	2.00 ± 1.73^{a}	51.7 ± 0.58^{a}	25.21 ± 0.82^{GH}	6.41	80.00
T9	2	100	40	32.20 ± 11.60^{D}	27.0 ± 9.90^{BCD}	3.33 ± 1.53^{a}	51.0 ± 4.32^{a}	30.31 ± 0.61^{DEFG}	24.09	90.91
T10	2	150	5	31.04 ± 8.43^{D}	33.5 ± 3.54^{ABCD}	0.67 ± 1.15^{a}	33.5 ± 3.54^{b}	34.63 ± 2.18^{BCD}	20.83	72.50
T11	2	200	28	48.18 ± 7.58^{ABCD}	22.0 ± 0.69^{CD}	1.33 ± 1.53^{a}	40.0 ± 5.62^{ab}	30.56 ± 1.01^{DEF}	24.44	78.79
T12	2	250	17	42.23 ± 14.61^{ABCD}	18.0 ± 2.23^{D}	0.67 ± 0.58^{a}	51.0 ± 2.66^{a}	36.70 ± 0.72^{BC}	7.11	70.59
ck_1	0	—	—	35.56 ± 8.43^{D}	27.0 ± 10.39^{BCD}	1.33 ± 1.53^{a}	43.0 ± 2.83^{ab}	37.56 ± 0.91^{AB}	9.33	100.0
ck_2	1	—	—	35.16 ± 7.56^{D}	29.0 ± 5.42^{BCD}	1.33 ± 0.58^{a}	50.0 ± 5.66^{a}	17.96 ± 0.30^{I}	13.51	100.0
ck_3	2	—	—	35.11 ± 4.32^{D}	27.0 ± 10.11^{BCD}	0.83 ± 1.04^{a}	33.33 ± 5.51^{b}	29.67 ± 0.17^{DEFG}	20.16	73.08

No.	X_1	X_2	X_3	Y_8	Y_9	Y_{10}	Y_{11}	Y_{12}	Y_{13}
T1	0	100	28	2.50 ± 0.22^{AB}	0.74 ± 0.04^{AB}	0.529 ± 0.01^{C}	2.86 ± 0.88^{CD}	1.23 ± 0.13^{AB}	273.29 ± 9.91^{A}
T2	0	150	17	1.60 ± 0.33^{C}	0.77 ± 0.07^{AB}	0.797 ± 0.01^{B}	1.98 ± 1.07^{D}	1.20 ± 0.07^{AB}	127.07 ± 7.51^{EFG}
T3	0	200	40	2.44 ± 0.44^{AB}	0.72 ± 0.08^{AB}	0.192 ± 0.01^{BF}	8.10 ± 2.83^{BCD}	1.11 ± 0.13^{ABC}	152.04 ± 2.80^{DEF}
T4	0	250	5	2.04 ± 0.27^{ABC}	0.78 ± 0.10^{AB}	0.113 ± 0.01^{E}	6.76 ± 3.40^{BCD}	1.53 ± 0.25^{A}	239.16 ± 31.19^{AB}
T5	1	100	5	1.78 ± 0.41^{ABC}	0.89 ± 0.07^{A}	2.447 ± 0.11^{A}	0.64 ± 0.00^{D}	1.09 ± 0.46^{ABC}	240.40 ± 11.44^{AB}
T6	1	150	28	1.52 ± 0.26^{C}	0.81 ± 0.10^{AB}	0.222 ± 0.03^{E}	6.88 ± 0.09^{BCD}	0.70 ± 0.09^{BC}	165.20 ± 8.05^{CDE}
T7	1	200	17	1.72 ± 0.23^{BC}	0.80 ± 0.07^{AB}	0.430 ± 0.02^{D}	3.81 ± 2.13^{BCD}	1.24 ± 0.07^{ABC}	189.02 ± 22.58^{CD}
T8	1	250	40	1.63 ± 0.65^{C}	0.78 ± 0.02^{AB}	0.117 ± 0.01^{FG}	12.29 ± 4.03^{BCD}	0.52 ± 0.07^{C}	61.91 ± 15.70^{H}
T9	2	100	40	1.88 ± 0.15^{ABC}	0.75 ± 0.09^{AB}	0.126 ± 0.01^{FG}	11.51 ± 2.23^{BCD}	1.44 ± 0.11^{A}	109.73 ± 5.14^{FGH}
T10	2	150	5	2.02 ± 0.45^{ABC}	0.83 ± 0.14^{AB}	0.100 ± 0.01^{G}	14.79 ± 2.69^{AB}	1.66 ± 0.17^{A}	94.80 ± 35.82^{GH}
T11	2	200	28	2.54 ± 0.40^{A}	0.76 ± 0.06^{AB}	0.061 ± 0.004^{G}	24.49 ± 11.68^{A}	1.52 ± 0.51^{A}	109.82 ± 25.34^{FGH}
T12	2	250	17	2.50 ± 0.60^{ABC}	0.65 ± 0.04^{B}	0.113 ± 0.005^{FG}	14.11 ± 4.49^{ABC}	1.22 ± 0.04^{AB}	88.76 ± 42.09^{GH}
ck_1	0	—	—	1.62 ± 0.23^{C}	0.92 ± 0.13^{A}	0.381 ± 0.02^{D}	3.81 ± 2.14^{BCD}	1.24 ± 0.07^{AB}	210.27 ± 6.52^{BC}
ck_2	1	—	—	1.72 ± 0.23^{BC}	0.80 ± 0.07^{AB}	0.430 ± 0.02^{D}	3.81 ± 2.14^{BCD}	1.14 ± 0.24^{ABC}	189.02 ± 22.59^{CD}
ck_3	2	—	—	1.82 ± 0.24^{ABC}	0.84 ± 0.15^{AB}	0.723 ± 0.03^{B}	2.26 ± 1.63^{CD}	1.04 ± 0.43^{ABC}	152.04 ± 9.29^{DEF}

No.: treatment number; X_1: seed soaking time in water (days); X_2: ESF intensity (kV·m^{-1}); X_3: ESF treatment time (min); Y_1: seed relative electroconductivity (%); Y_2: initiation time of seed germination under light conditions (days); Y_3: germination percentage of seeds under light conditions (%); Y_4: initiation time of seed germination under low temperature (days); Y_5: germination percentage of seeds under low temperature (%); Y_6: seedling emergence percentage (%); Y_7: seedling survival percentage (%); Y_8: seedling height (cm); Y_9: seedling root-collar diameter (mm); Y_{10}: SOD activity (U·mg^{-1} protein); Y_{11}: soluble protein contents (mg·g^{-1} FW); Y_{12}: total chlorophyll content (mg·g^{-1} FW); Y_{13}: total soluble sugar content (mg·g^{-1} FW). Letters are Duncan grouping value: same letter showed no significant difference; capital letters showed significant difference at 0.01 level; lowercase letters showed significant difference at 0.05 level. The data were mean value of 3 replicates and the number behind "±" was standard difference.

Effect of electrostatic field treatments on water absorbing ability of dry seeds

All ESF treatments could enhance water absorbing ability of dry seeds of *S. pohuashanensis* (Fig. 1). Treatment 2 (150 kV·m^{-1} for 17 min) and treatment 3 (200 kV·m^{-1} for 40 min) improved the water absorbing ability of seeds more evident than others, but there was no significant difference between all ESF treatments and control (p=0.565).

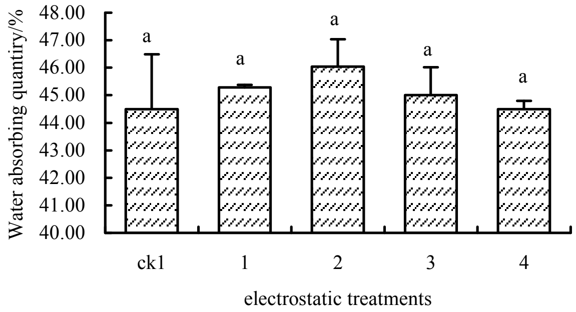

Fig.1 Effect of electrostatic treatment on water absorbing percentage of *S. pohuashanensi* seeds. Values represent the mean and standard error, letters are Duncan grouping value, and the same letter showed no significant difference.

Effect of electrostatic field treatments on electroconductivity of seeds

Effects of seed soaking time (days) in water (X_1), ESF intensity (kv·m^{-1}) (X_2), ESF treatment time (min) (X_3) on seed relative electroconductivity (%) (Y_1) is shown as the following linear regression equation:

$$Y_1 = 51.31 - 10.69X_1 + 0.03X_2 - 0.04X_3 \tag{2}$$

(R=0.802, F value =4.819, p=0.033<0.05)

The equation indicated that every treatment factor had significant effect on seed relative electroconductivity. Path analysis shows that the most important factors affecting seed relative electroconductivity (direct path coefficient was -0.785) is seed soaking time, followed by ESF intensity (direct path coefficient 0.162), and ESF treatment duration (direct path coefficient -0.043).

ANOVA result showed that ESF treatments had significant effect on the relative electroconductivity of seeds (p=0.0003<0.01).

Effect of electrostatic field treatments on seed germination

Germination initiation time and germination percentage of seeds germinated under light in room temperature

Quadratic polynomial regression equation for seed germination initiation time under light (Y_2) and seed soaking time (days) in water (X_1), ESF intensity (kV·m^{-1}) (X_2), ESF treatment time (min) (X_3) is as follow:

$$Y_2 = 87.48 + 18.26X_1 - 0.50X_2 - 0.10X_3 - 14.00X_1^{\,2} + 0.001X_2^{\,2} + 0.004X_3^{\,2} \tag{3}$$

(R=0.952, F value =8.136, p=0.018<0.05)

Optimization analysis by the regression equation showed that the shortest germination initiation time under light at room temperature for the treated *S. pohuashanensi* seed was 20 days. The treatment of shortest germination initiation time occurred when the seeds were soaked for two days (X_1) and then treated with 201 kV·m^{-1} (X_2) ESF for 30 min (X_3). Path analysis shows that seed soaking time is the most important factor affecting seed germination initiation time (direct path coefficient was 0.650), followed by ESF intensity (direct path coefficient 0.375), and ESF treatment duration (direct path coefficient -0.024).

No significant level regression equation can be developed for seed germinate percentage (Y_3), seed soaking time (day) in water (X_1), ESF intensity (kV·m^{-1}) (X_2), and ESF treatment time (min) (X_3).

ANOVA result showed that the ESF treatments had no significant effects on seed germination percentage under light (p=0.533>0.05).

Germination initiation time and germination percentage of cold stratificated seeds

No significant level regression equation can be developed for germination initiation time (Y_4) and germination percentage (Y_5) of seeds cold stratified after ESF treatments and seed soaking time (d) in water (X_1), ESF intensity (kV·m^{-1}) (X_2), ESF treatment time (min) (X_3) (p>0.05).

ANOVA result showed that ESF treatments had significant effect on the seeds germination initiation time (p=0.02<0.05), the seeds germination initiation time for no soaking and soaking one day was longer than controlling time, but this difference did not reach significant level (p > 0.05). After soaking two days, the electrostatic intensity (100 kV·m^{-1}) on seeds for 40 min and the electrostatic intensity (250 kV·m^{-1}) on seeds for 17 min all significantly delayed germination initiation time on seeds of *S. pohuashanensi* (p < 0.05).

ANOVA result showed that ESF treatments had a highly significant effect on the seeds germination percentage (p=0<0.01). Soaking one day after electrostatic field treatment on seeds indicated a highly significant germination percentage (p < 0.01) at low temperature. Soaking two days after electrostatic field treatment on seeds improved germination percentage at low temperature. The germination percentage of treatment No.10 (soaking two days, 150 kV·m^{-1} treating for 5 min) and treatment No.12 (soaking two days, 250 kV·m^{-1} treating for 17 min) were significantly higher than control (p=0.007 and 0.001). For seeds not soaked, only the seeds on electrostatic intensity (200 kV·m^{-1}) for 40 min had higher germination percentage than control (p=0.016) (Fig. 2–4).

Effect of electrostatic field treatment on seedling after sowing

Linearity regression and Quadratic polynomial regression curve-fitting were carried out for the effects of each factor on germination of seeds Y_6 and survival rate Y_7, and the results of regression were not significant (p > 0.05). ANOVA result revealed that germination percentage and survival rate showed no significant difference with different treatments (p=0.500 and 0.330 respectively), suggesting that electric field treatment had no significant effect on germination of seeds and subsequent survival of seedlings.

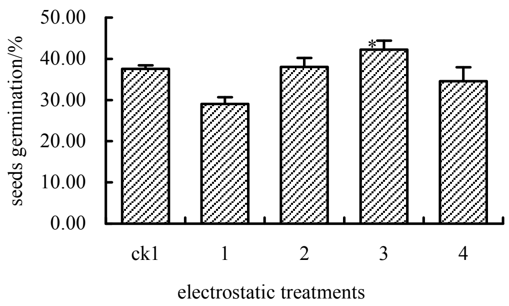

Fig. 2 Effect of electrostatic field treatment and low temperature on the seed germination of *S. pohuashanensi*. Values represented the mean and standard error, *showed significant difference at 0.05 level than control.

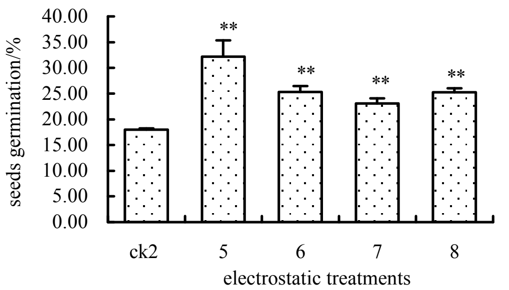

Fig.3 Effect of electrostatic field treatment and low temperature on the seed germination of *S. pohuashanensi* after soaked seeds in water for one day. Values represented the mean and standard error, **showed significant difference at 0.01 level than control.

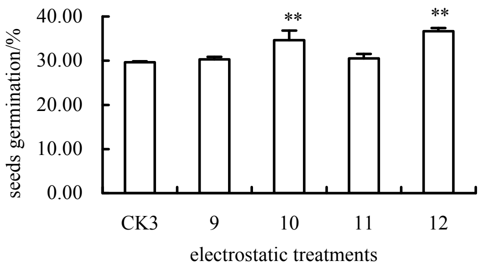

Fig.4 Effect of electrostatic field treatment and low temperature on the seed germination of *S. pohuashanensi* after soaked seeds in water for two days. Values represented the mean and standard error, **showed significant difference at 0.01 level than control.

Effect of electrostatic field treatment on seedling height and diameter

Seedling diameter and height were tested after 55 days of sowing when the seeds grew into seedlings with four leaves and a curve leaf. Linearity regression and Quadratic polynomial regression of each factor on germination seeds for the height of Y_8 and the diameter of Y_9 were carried out, but curve-fitting results showed that the regression was not significant (p > 0.05).

ANOVA result shows that ESF treatments have a highly significant effect on the seedling diameter (p=0<0.01). There was no significant difference between the diameter of seedlings derived from seeds soaking for one day after various electrostatic field treatments and that derived from control (p > 0.05), but the diameter of seedlings derived from seeds without soaking and seeds soaking for two days after electrostatic field treatment was lower than that derived from control. The diameter of seedlings derived from seeds of treatment No.1 (without soaking, 100 $kV \cdot m^{-1}$ for 28 min), treatment No.3 (without soaking, 200 $kV \cdot m^{-1}$ for 40 min) and treatment No.12 (soaking two days, 250 $kV \cdot m^{-1}$ for 17 min) was significantly less than that derived from control (p < 0.05, p < 0.05 and p < 0.01 respectively).

ANOVA result shows that ESF treatments have significant effect on the seedling height (p=0<0.01). Height of seedlings derived from seeds without soaking and seeds soaking for two days were significantly higher than that of control, in which heights of seedlings derived from seeds of treatment No. 1 (without soaking, 100 $kV \cdot m^{-1}$ for 28 min), treatment No.3 (without soaking, 200 $kv \cdot m^{-1}$ for 40 min), treatment 4 (without soaking, 250 $kv \cdot m^{-1}$ for 5 min) and treatment No.11 (soaking two days, 200 $kv \cdot m^{-1}$ for 28 min) was much more higher than that of control (p < 0.05). Height of seedling derived from seeds soaking for one day was lower than that form control, but the difference does not reach the significant level (p >0.05).

This result indicated that electrostatic field and low temperature treatments could improve seed germination of *S. pohuashanensi*, but they had no evident effect on seedling diameter growth. For seeds without soaking and seeds soaking for two days, proper electrostatic field treatments could improve seedling height growth (Table 3).

Effect of electrostatic field treatment on physiological metabolism of seedling

SOD activity in seedling leaf

SOD is the key of plant oxygen metabolite and can usually be induced. The changes in outer environment could have effect on SOD activity. The linear regression equation of the factor X_i (i=1, 2, 3) that affects the SOD activity in leaves was defined as:

$$Y_{10} = 1.472 - 0.677X_1 - 0.009X_2 - 0.030X_3 \quad (4)$$

(R=0.835, F value =6.139, p=0.018<0.05).

This showed that every factor significantly affected the SOD activity in seedling leaves. Path analysis indicates that the most

important factors affecting SOD activity is soaking time X_1 (direct path coefficient -0.552), followed by electrostatic intensity X_2 (direct path coefficient -0.486) and treatment time X_3 (direct path coefficient -0.393).

ANOVA result shows that there is significant difference between the electrostatic field treatments on SOD activity (p=0<0.01). SOD activities of treatment No.1 (no soaking, 100 kV·m^{-1} for 28 min), treatment No. 2 (no soaking, 50 kV·m^{-1} for 17 min) and treatment No.5 (soaking one day, 100 kV·m^{-1} for 5 min) were significantly higher than that of control (p < 0.01), while the SOD activities of other treatments were significantly lower than that of control (p<0.01). Changing activity of SOD in leaf has a significant negative correlation to total soluble sugar content, whereas, changing total soluble sugar content brings out a significant positive correlation to seedling diameter (Table 3).

Soluble protein contents in seedling leaf

Protein standard curve is Y=0.0007X-0.01 (R=0.959). Effect of the factor X_i (i=1, 2, 3) on soluble protein content Y_{11} could be defined as the linear regression equation:

$$Y_{11} = -4.776 + 5.480X_1 + 0.041X_2 + 0.020X_3 \quad (5)$$

(R=0.826, F value =5.706, p=0.022<0.05).

This showed that every factor had significant effect on soluble protein content in seedling leaf. Path analysis shows that the most important factors affecting soluble protein content is soaking time X_1 (direct path coefficient 0.733), followed by electrostatic intensity X_2 (direct path coefficient 0.377) and treatment time X_3 (direct path coefficient 0.042).

ANOVA result shows that there is significant difference between the electrostatic field treatments on soluble protein content (p=0<0.01). Soluble protein content in seeds soaked for two days after electrostatic field treatment was significantly higher than that in control (p <0.05). Soluble protein content in leaf brings out a significantly negative correlation to total soluble sugar content, SOD activity and seedling diameter, whereas brings out a significant positive correlation to seedling height (Table 3).

By comprehensive analysis of the effects of electrostatic field treatment on SOD activity and on soluble protein contents in seedling leaf, we induced the reason of bringing down activity of SOD in leaf is not the cause from protein synthetic amount in plant, but it is caused from reducing concentration of oxygen free radical (O^{2-}). Electrostatic field treatments can improve total soluble sugar content in leaf of *S. pohuashanensis* and reduce oxygen free radical (O^{2-}) for generation in chloroplast and significantly decreases activity of SOD, which is the key enzyme of eliminate oxygen free radical. This will benefit the plants growth, postponing plant decrepitude and the hardiness ability.

Table 3. Correlation analysis among growth targets of *S. pohuashanensis* seedlings

	SOD activity (mg·g^{-1}FW)	Soluble protein content (U·mg^{-1}protein)	Total Chlorophyll content (mg·g^{-1})	Soluble sugar content (mg·g^{-1})	Root-collar Diameter (mm)	Seedling height (cm)
SOD activity	1					
Soluble protein content	-0.621**	1				
Total chlorophyll content	-0.120	0.300	1			
Soluble sugar content	0.521**	-0.661**	0.106	1		
Root-collar diameter	0.618**	-0.310*	-0.083	0.279	1	
Seedling height	-0.235	0.355*	0.521**	0.250	-0.396**	1

Total chlorophyll in seedling leaf

Effect of the factors X_i (i=1, 2, 3) on the total chlorophyll Y_{12} could be defined as the linear regression equation:

$$Y_{12} = 1.281 + 0.086X_1 - 0.0007X_2 - 0.007X_3 \quad (6)$$

(R=0.780, F value=4.150, p= 0.048<0.05),

which shows that every factor has a significant effect on the total chlorophyll in seedling leaves.

Path analysis shows that the most important factors affecting the total soluble sugar content is treatment time X_3 (direct path coefficient-0.586), followed by soaking time X_1 (direct path coefficient 0.443), and electrostatic intensity X_2 (direct path coefficient-0.259).

ANOVA result shows that there is a highly significant difference between the effects of electrostatic field treatments on the total chlorophyll content in young sprout leafs (p=0.001<0.01). Among the treatments, treatment No.10 (immersed to grow for two days, the 150 kV·m^{-1} for 5 min) was the best, resulting in highest total chlorophyll content. The total chlorophyll content has a strong positive correction with seedling height (Table 3), suggesting that the electrostatic field treatment could increase the photosynthetic ability of *S. pohuashanensis* seedling and promote the growth of seedling of *S. pohuashanensis*.

Soluble total sugar contents in seedling leaf

Glucose standard curve: Y = 0.001X + 0.006 (R = 0.991). Effects of factors X_i (i =1, 2, 3) on total soluble sugar contents Y_{13} could be defined as the linear regression equation:

$$Y_{13} = 18.774 - 1.936X_1 - 0.018X_2 - 0.072X_3 \quad (7)$$

(R=0.795, F value=4.851, p=0.038<0.05),

which shows that every factor has a significant effect on the total soluble sugar content in seedling leaf. Path analysis shows that

the most important factors affecting the total soluble sugar contents is soaking time X_1 (direct path coefficient-0.603), followed by electrostatic intensity X_2 (direct path coefficient -0.376) and treatment time X_3 (direct path coefficient -0.355).

ANOVA shows that there is significant difference between the electrostatic field treatments on the total soluble sugar content (p=0<0.01). Total soluble sugar content for the seeds soaked two days after electrostatic field treatment was significantly lower than that of control ($p < 0.05$). Total soluble sugar contents in treatment No.1 (without soaking, 100 kV·m^{-1} for 28 min) and treatment No. 5 (soaking one day, 100 kV·m^{-1} for 5 min) were significantly higher than that of control ($p < 0.05$), whereas total soluble sugar contents in treatment No.2 (no soaking, 150 kV·m^{-1} for 17 min), treatment No. 3 (no soaking, 200 kV·m^{-1} for 40 min) and treatment No. 8 (soaking one day, 250 kV·m^{-1} for 40 min) were significantly lower than that of control ($p < 0.05$). There were no significant difference in total soluble sugar content of all other treatments and control. Soluble protein content in leaf has a negative correlation to total soluble sugar content, whereas total soluble sugar content brings out a positive correlation to SOD activity (Table 3).

Conclusion and discussion

All the electrostatic field treatments promoted the absorbing water ability of dry seeds. After ESF treatment, germination initiation time of seeds was shortened at room temperature and under light. Cold stratification after electrostatic field treatment could also promote germination. Electrostatic field treatment could increase seedling height, significantly affect SOD activity, and significantly improve soluble protein content in leaf, total soluble sugar content and total chlorophyll in leaf. That is, electrostatic field treatment is effective way to improve seed viability and germination, seedling resistance, photosynthetic ability and subsequent growth of seedlings of *S. pohuashanensis*.

Soaking time has a significant effect on seed relative electroconductivity, seed germination under light, SOD activity, soluble protein content, and total soluble sugar content of seedling leaves. ESF intensity exerts a moderate effect on these indexes. ESF treatment time only has strong effect on total chlorophyll content, but has no evident effects on other indexes.

The organisms on earth are inevitably affected by the enormously strong electrostatic field including plants. The synergy effect between electrostatic field and other environment physical factors promotes the establishment of an ordered constitution inside a plant, such as enzyme arranged in the cell, chloroplast's thylakoid membrane states, electron flowing along the root surface in a way of symmetry and starch arranged in the cell. These activities ensure the maintenance of a balance state in plants and each plant's exercising function. By changing the Electrostatic Field around a plant, the electric motion in the plant can be changed, which results in the significantly changing in physiological and biochemical reactions. Treating pollen, seeds or seedlings with high-intensity electrostatic field would result in the acceleration of germination, the promotion of growth and increase of both yield and quality (Li et al. 1996; Qiao et al. 1998; Cai et al. 2003). For example, proper electrostatic field treatment can increase the vigor of *Pinus masson* and *P. thunbergii* seeds to result in fast germination (Zheng and Zhu, 1998). Zhao et al. (1998) and Zhu et al. (2000) suggested that electrostatic field treatment could increase the vigor of radical scavenging enzyme system and basic metabolic enzyme. Morar et al (1999) demonstrated that exposure to a high-intensity electrostatic field can be an effective substitute for the chemical agents. The experiments were carried out on bean seeds (*Phaseolus vulgare*). Under the normal conditions (no treatment) only about 30% of a reference sample of such seeds germinated. Other samples were subjected to 50 Hz electrostatic fields ranging from 2 to 16 kV·cm^{-1} with exposure time ranging from 1 to 30 s. In the optimum laboratory test, more than 99% of the seeds germinated. The field tests proved the efficiency of this method, which could be successfully employed for the prevention or treatment of various seed-transmitted diseases of plants. Ozone generation by partial discharges between seeds seems to be the main sterilizing agent, while the activation of OH radicals under the action of the high-intensity electrostatic field may explain the intensification of the biological processes. In our study, electrostatic field treatment did not significantly promote the germination of *S. pohuashanensis* seeds under room temperature in light. Whereas, the combined treatment of electrostatic field and cold stratification significantly promoted the germination of *S. pohuashanensis* seeds (to 42.20%), indicating a relationship between germination ability and the characteristics of seeds (the deep dormancy nature, which requires a 0–5°C stratification to overcome). The increase in SOD activity, soluble protein contents, total chlorophyll and total soluble sugar content indicated that electrostatic field treatment could improve the resistance of *S. pohuashanensis* seedlings.

The uniform design is a multilevel test design with the advantages of fewer experiments, uniform distribution and well representative, which made it an important way to optimize experimental parameters and techniques (Song et al. 2000). Regression analysis was a critical mean of uniform design data analysis (Tang 2002). Significance and degree of fitting of the equation should be tested after its establishment to determine the reliability of the relationships. In the present study, the regression analysis as well as liner regression analysis was carried out for the correlations of soaking time, electrostatic field intensity, and electrostatic field treatment duration with seed germination initiation time, germination percentage, emergence rate of seedling, survival rate of seedling, seedling height, and root-collar diameter; however, significance tests show that there is no significant correlation between the affecting factors and seed indexes. Thus, optimization of the parameters and analyses of the factors could not be carried out according to the regression equation. It was induced that it might be other factors (the physiologic after-ripening of seeds) relating to above indexes. Accordingly, we could only use conventional analysis of ANOVA for electrostatic field treatment and these indexes. We suggested that three factors in the equation should be further studied for their effects on the results. Due to the limit of the types of electrostatic field inten-

sity and the treating duration available, the selected condition could only be regarded as a relative condition. And expanding the range of electrostatic field intensity and treating duration might lead to more reasonable results.

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